## ABSTRACT

The present invention is directed to Protein-fragment Complementation Assays (PCAs) and assay compositions based on fluorescent proteins. The invention provides methods for fragmenting fluorescent proteins and generating mutant fragments with desired spectral characteristics for PCA. The invention encompasses assays and compositions based on fluorescent proteins from the species Aequorea, Anemonia and Anthozoa. In particular, the invention is directed to fragments of mutant fluorescent proteins having improved spectral properties over the wild-type proteins. The invention encompasses fragments of mutant versions of A. Victoria green fluorescent protein (GFP), in particular yellow fluorescent proteins (EYFP and super-EYFP), 'Venus', cyan, 'citrine', blue, cyan-green, and photoactivatable variants of GFP The invention also encompasses red fluorescent PCAs based on Discosoma red fluorescent protein (RFP PCA)and a kindling fluorescent protein PCA (KFP1 PCA) derived from Anemonia sulcata. Any useful mutation of a fluorescent protein can be engineered into a fragment, generating a wide range of assays useful for drug discovery, target validation, high-throughput screening, high-content screening, pathway mapping, drug mechanism-of-action studies, biosensors, and diagnostics.